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Relationship between hardness and myowater properties in Wooden Breast affected chicken meat: A nuclear magnetic resonance study

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ABSTRACT

The role of myowater-holding on the development of the hardness of Wooden Breast (WB) affected chicken breasts was investigated. Transverse (T_2) relaxation times and proportions of myowater populations (T_{2B}, T_{21} and T_{22}) were assessed using low-field nuclear magnetic resonance (NMR) relaxometry and integrated with meat compression measurements. Two muscle conditions (M: Normal (N) vs WB), four sampling locations (L), four sampling times (T) and interactions (M x L and M x T) were considered. Compared to N, WB was harder, the extramyofibrillar myowater population (T_{22}) was increased and the relaxation time of the water trapped into the myofibrillar matrix (T_{21}) was also increased. A link between the T_{21} relaxation time of water trapped into the myofibrillar matrix and hardness was suggested for the WB muscles. During storage, a redistribution of water occurred over time, as revealed by an increasing trend of the T_{21} population, but a concomitant texture evolution did not reflect this change. The cranial/superficial part of the breasts exhibited the highest amount of the extramyofibrillar water population (T_{22}), and the texture of this muscle part was harder than the deep layers. However, the role of myowater on muscle hardness was not fully clarified by this study.

1. Introduction

An emergent myopathy in fast growing, meat-type broiler chickens has been described and named Wooden Breast (WB) (Sihvo, Immonen, & Puolanne, 2014). Macroscopically, the affected *Pectoralis major* muscle is hard, pale, outbulging and sometimes superficially covered with small haemorrhages, exudate and occasionally White Striping; extended areas with poor cohesion of the muscle bundles are also visible beneath the lesioned areas (Dalle Zotte et al., 2017). Histologically, the condition was defined as a moderate or severe polyphasic myodegeneration with regeneration of the muscle tissue; therefore, inflammation and necrosis are detected and are followed by accumulation of a variable amount of interstitial connective tissue (fibrosis) as reparative response (Sihvo et al., 2014). Starting from the first surveys on WB and until more recent studies, the fibrotic response has been considered as the primary factor for the typical hardness of the affected tissue (Clark & Velleman, 2016; Sihvo et al., 2014), as extensive hard consistency of raw meat and increased intramuscular collagen often occur together (Chatterjee, Zhuang, Bowker, Rincon, & Sanchez-Brambila, 2016; Petracci et al., 2015; Sihvo et al., 2014; Soglia et al., 2016a). Interestingly, it was demonstrated that WB muscle does not possess a homogeneous structure, as fibrosis was found to affect the anterior portions of the fillets, whereas the middle-ventral and postero-ventral locations were less or not at all affected (Clark & Velleman, 2016). In addition, contrary to normal breast muscles, also superficial and deep layers in raw affected *Pectoralis major* muscle has been found to differ in terms of textural properties, which probably reflects a variation in the muscle architecture between layers. Indeed, according to Gao (2015), hard consistency of the muscle is mainly present at the surface level at the early *post mortem* stage and becomes as soft as the normal condition during a chilled storage. As previously mentioned, fibrosis has been considered the major reason for the hard texture of affected chicken breasts so far. However, some cases of hard breasts without a significant accumulation of collagen were detected in broiler chickens both in very young birds (Sihvo, Lindén, Airas, Immonen,

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& Puolanne, 2017a; Sihvo et al., 2017b) and at commercial slaughter age (Dalle Zotte et al., 2017; Sihvo et al., 2014), thus opening new hypotheses on the reasons for muscle hardness development. One hypothesis may involve the role of myowater. Indeed, changes in muscle microstructure directly affect water distribution among the three water populations defined by nuclear magnetic resonance (NMR) T_2 relaxation studies: T_{2B} (H_2O closely associated to macromolecules/proteins), T_{21} (H_2O trapped into myofibrillar matrix) and T_{22} (extramyofibrillar H_2O), with each water compartment exhibiting its typical relaxation time (Bertram, Purslow, & Andersen, 2002; Bertram et al., 2001). In a previous study of Soglia, Laghi, Canonico, Cavani, and Petracci (2016b), the WB condition resulted in a remarkable decrease in the intramyofibrillar fraction and a concomitant increase in the extramyofibrillar water fraction. Consequently, the present study aimed to investigate the role of the chemical-physical state of myowater on hardness in WB affected chicken meat, integrating low-field NMR relaxometry with texture analysis (compression test). NMR properties and hardness were evaluated not only according to muscle condition but also considering a 72 h *post mortem* chilled storage and four different sampling locations, as these factors have been shown to affect meat textural properties (Soglia et al., 2017).

2. Material and methods

2.1. Samples collection and preparation

During two different sampling times, a total of 96 breast muscles from 34-day-old broiler chickens were collected at a commercial Danish slaughterhouse (Danpo A/S, Aars, Denmark). Each time, 48 fillets were selected according to the presence or the absence of severe Wooden Breast lesions, thus obtaining 24 macroscopically unaffected or normal (N) and 24 Wooden Breast (WB) samples. The selection based on the visual and palpatory inspection of *Pectoralis major* muscles; breasts exhibiting diffused hardened areas were scored as WB. The presence of bulges, pale colour and the surface covered with exudate, haemorrhages and white striping were also detected in the selected WB breasts (Dalle Zotte et al., 2017; Sihvo et al., 2014). On the contrary, fillets with soft and elastic tissue with uniform colour were scored as N. After selection, breasts were immediately packed into polyethylene bags, kept cool and transported to the NMR laboratory of the Department of Food Science (Aarhus University, Årslev), where further analyses took place. At the laboratory, fillets were kept at 4 °C for four different storage times: 10, 24, 48 and 72 h *post mortem* (*pm*); 6 WB-affected (WB) and 6 normal (N) fillets were used for each time. Four stripes per breast (1 cm × 1 cm x 4 cm, 5 ± 0.5 g) were excised parallel to the fibre direction; two of them were obtained from the cranial end of the fillet (CRA), whereas the other two were cut from the medial portion (MED). Within each portion, one stripe was snipped from the superficial layer (S: 0.2–1.2 cm deep under the muscle surface) and the other was snipped from the deep layer (D: 1.5–2.5 cm deep under the muscle surface) (Gao, 2015; Soglia et al., 2017). Locations were named as follows: CRA/S = cranial/surface; CRA/D = cranial/depth; MED/S = medial/surface; MED/D = medial/depth (Fig. 1). Accordingly, 24 + 24 meat samples were prepared and analysed per each of the eight measurement days.

2.2. NMR measurements

Meat stripes (1 cm × 1 cm x 4 cm) were placed in glass test tubes, which were sealed with paraffin film and thermostated at 25 °C for 20 min in a waterbath. Thereafter, transverse relaxation time (T_2) measurements were performed on a Maran Benchtop Pulsed NMR Analyser (Resonance Instruments Ltd, Witney, UK)

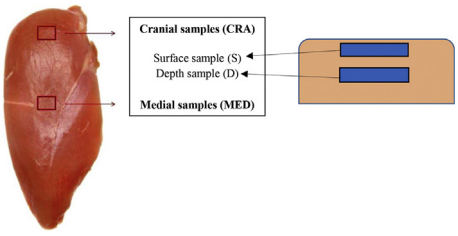

Fig. 1. Sampling locations.

equipped with a 18 mm probe head operating at a magnetic field strength of 0.47 T and a corresponding resonance frequency for protons of 23.2 MHz. Transverse relaxation time (T_2) was measured using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence (Carr & Purcell, 1954; Meiboom, Gill, Huxley, & Niedergerke, 1958) and a τ-value (time between 90 and 180° pulse) of 150 μs Data from 4096 echoes were acquired as 16 scan repetitions. The obtained T_2 data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, and Dawson (1981) and carried out in MatLab (The Mathworks Inc., Natick, MA, USA) using in-house scripts. Plots of relaxation amplitudes for individual relaxation processes vs relaxation times revealed the presence of three relaxation populations. For each of the three water populations T_{2B}, T_{21} and T_{22}, relaxation times were calculated from the peak position and proportions of protons exhibiting those relaxation times were calculated from the corresponding area under each peak, using an in-house programme written in Mathlab (The Mathworks Inc., Natick, MA, USA).

2.3. Hardness measurements

After the NMR analyses, the same meat samples were then subjected to a single compression test in order to determine their hardness. A Brookfield CT3 texturometer (Texture Technologies Co, Hamilton, MA, USA) equipped with a 250 N load cell was used, setting the trigger load at 0.3 N and the test speed of at 50 mm/min (0.83 mm/s). One sample from each muscle (48) was taken at each storage time (10, 24, 48 and 72 h *pm*). Strips were cut before analysis (1 cm × 1 cm x 3 cm) in order to fit to the compression probe (1 cm^2) and to the measuring cell (1 cm × 1 cm x 3 cm), which were modified according to Lepetit and Culioli (1994) and Campo, Sañudo, Panea, Alberti, and Santolaria (1999). Strips were compressed to 80% of their initial height (Lyon & Lyon, 2001) perpendicularly to the fibre direction, which, therefore, could extend only longitudinally. From each measurement a curve was obtained, and the peak force represented the maximum hardness (expressed in newtons).

2.4. Statistical analysis

The “Location” (L) effect was created combining breast portion (CRA/MED) and layer (S/D); four locations resulted from the combinations (CRA/S; CRA/D; MED/S; MED/D). Then, data were analysed using a SAS 9.1.3 statistical software package for Windows (SAS, 2004). Variables were evaluated by ANOVA, choosing a mixed model (PROC MIXED) which considered muscle condition (M: N; WB), location (L), hour *pm* (T: 10 h, 24 h, 48 h, 72 h) and sampling time (1; 2) as fixed effects, whereas sample (breast) was considered as random repeated effect. The interactions M x T and M x L were also studied. Post-hoc pairwise comparisons were evaluated by Bonferroni adjustments. Pearson correlations between hardness and NMR variables were performed with the two muscle conditions separately considered. Two significance levels were assigned:

$P \leq 0.05$ and $P \leq 0.01$.

3. Results and discussion

The present study investigated the role of the physico-chemical state of myowater on the development of hardness in Wooden Breast affected chicken breast muscles by NMR relaxometry and texture analysis (compression test). The considered variables were affected by muscle condition (M), storage time (T), sampling location (L) and their interaction (M x T and M x L). In Table 1, the overall effects of the main variable (muscle condition; WB or N) on NMR relaxation times and hardness across all other variables are presented. WB breasts were characterized by longer T_{2B} and T_{21} relaxation times than N breasts (T_{2B}: 0.82 vs 0.50 ms; T_{21}: 21.0 vs 13.6 ms; $P < 0.01$). The proportions of T_{2B} (the water closely associated to macromolecules) and T_{21} (water trapped into the myofibrillar matrix) populations were lower in WB than in N samples (T_{2B}: 4.47 vs 5.29%; T_{21}: 88.5 vs 93.4%, $P < 0.01$) in favour of extramyofibrillar water, T_{22} population (7.05 vs 1.26%, $P < 0.01$). WB breasts clearly experienced changes in muscle structure, and structural differences affect water mobility and distribution as detected by NMR T_2 relaxometry (Bertram et al., 2002). This can be ascribed to the fact that T_2 relaxation will reflect water-protein interactions and thereby also depend on spatial factors as these will influence the probability of a water molecule to meet a protein acting as a relaxation sink (Hills, Takacs, & Belton, 1990). In association with this hypothesis, it has been shown that a close relationship exists between meat structure and T_2 relaxation rate of water populations in meat (Bertram et al., 2002). Furthermore, structural features of intrinsic meat proteins can also influence T_2 relaxation through their ability to act as relaxation sinks. Thus, conformation changes in protein structures caused by pH variations and/or changes in exposure of hydrophobic groups are also reflected in the NMR T_2 relaxation pattern of meat (Bertram, Whittaker, Andersen, & Karlsson, 2003). Interestingly, sarcomere length as well as the tensile strength were found to increase in WB-affected breasts (Ababei, 2016; Tijare et al., 2016). Therefore, muscle tactile stiffness and contracted appearance in severe WB cases could not be related to a general sarcomere shortening. On the contrary, according to Gordon, Huxley, and Julian (1966), sarcomere stretching seemed to be related to an increase in muscular tension, and their relationship was described by a bell-shaped curve. At the same time, sarcomere length has been demonstrated to be highly correlated ($r = 0.84$) to the T_{21} relaxation time constant (Bertram et al., 2002), thus indicating that structural changes involving sarcomere length also affect the behaviour of this water population. Therefore, the present results suggest a connection relating muscle structure, the T_2 relaxation time of water trapped into the myofibrillar matrix and muscle hardness.

The correlation between compression and NMR relaxation times was studied within the two muscle conditions (Table 2). Interestingly, hardness was significantly ($P < 0.01$) correlated with T_{2B} ($r = 0.48$), T_{21} ($r = 0.46$) and T_{22} ($r = 0.41$) relaxation times only in the WB group. The analysis of correlation between texture and NMR relaxation times agreed with the statement of Pearce et al. (2008). They investigated the relationship occurring between shear force and NMR relaxation times in lamb *M. longissimus dorsi* during a storage period, and they found a relationship of $r = 0.78$ between NMR relaxation measurements and shear force. The data also suggest a positive correlation between the T_{21} relaxation time and the compression force, but only in WB meat.

Table 3 shows the effects of storage time on NMR relaxation times, water population percentages and muscle hardness. Hardness was not influenced by the interaction M x T ($P > 0.05$), similarly to Soglia et al. (2017), although a weak tendency for an aging-type tenderisation may be seen in both studies. The T_{21} relaxation times were generally longer in WB group than in N group, and the times did not change during the storage period within the groups.

The proportion of the population T_{2B} was not affected by the interaction M x T ($P > 0.05$), but for the T_{21} the overall M x T effect was significant ($P < 0.040$). The T_{21} population was in general higher in N samples than in the WB samples ($P = 0.01$), and for the N samples this water population remained stable during storage, while in the WB samples the values were higher in the 72 h samples than in the 10 h samples ($P = 0.01$). The T_{21} population was significantly higher in WB samples than the N ones throughout the storage period ($P = 0.01$), with the exception of WB 72 h, which was statistically similar to N 10 h. In addition, T_{22} percentages of WB samples decreased from 10 to 72 h *post mortem*, whereas the same fraction, being also lower than in WB, remained unchanged throughout the cold storage period in N samples ($P = 0.01$).

Straadt, Rasmussen, Andersen, and Bertram (2007) and Pearce et al. (2008) indicated for pork and sheep meats respectively, a tenderising action of storage in association with a redistribution of water, that was shown as a lower amount of the T_{22} population in favour of the intramyofibrillar compartment T_{21}. The observations of the current study on chicken meat did not agree with the observations collected for other meat species. Indeed, differently from

Table 1
Effect of the two muscle conditions (M: Wooden Breast, WB; Normal, N) on NMR T_2 relaxation times and populations percentages, and hardness. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). The table shows mean values of all breasts, which were analyzed at 10, 24, 48 and 72 h *pm* and sampled at the level of the four locations (CRA/S, CRA/D, MED/S, MED/D).

Variables	Muscle condition (M)		*P*-value
	WB	N	
No. of breasts	48	48	
Time constants (ms)			
T_{2B}	0.82 ± 0.03 ^{A}	0.50 ± 0.03 ^{B}	<0.01
T_{21}	21.0 ± 0.5 ^{A}	13.6 ± 0.5 ^{B}	<0.01
T_{22}	59.4 ± 3.1	65.7 ± 3.1	0.161
Populations (%)			
T_{2B}	4.47 ± 0.07 ^{B}	5.29 ± 0.07 ^{A}	<0.01
T_{21}	88.5 ± 0.3 ^{B}	93.4 ± 0.3 ^{A}	<0.01
T_{22}	7.05 ± 0.28 ^{A}	1.26 ± 0.28 ^{B}	<0.01
Hardness			
Compression (N)	24.8 ± 0.6 ^{a}	23.0 ± 0.6 ^{b}	<0.05

$^{a, b}$ Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$.
$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

Table 2
Correlations between NMR parameters (relaxation times and populations percentages) and hardness, evaluated considering the two muscle conditions (M: Wooden Breast, WB; Normal, N) separately. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). The table considers mean values of breasts analyzed at 10, 24, 48 and 72 h *pm*, and sampled at the level of the four locations (CRA/S, CRA/D, MED/S, MED/D).

Muscle condition	WB		N	
Variables	Compression (N)	*P*-value	Compression (N)	*P*-value
Time constants (ms)				
T_{2B}	0.48	<0.01	0.15	<0.05
T_{21}	0.46	<0.01	0.13	0.089
T_{22}	0.41	<0.01	−0.02	0.849
Populations (%)				
T_{2B}	−0.06	0.409	−0.22	<0.01
T_{21}	−0.21	<0.01	0.02	0.758
T_{22}	0.21	<0.01	0.18	<0.05

Correlations ≥ 0.40 were considered high and significant when $P \leq 0.05$.

Table 3
Effect of the interaction between muscle condition (M) and Time (T) on NMR T_2 relaxation times and population percentages, and hardness. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). Values of each time consider all the four locations together (CRA/S, CRA/D, MED/S, MED/D).

Muscle condition (M)	WB				N				*P*-value
Hours *p.m.* (T)	10	24	48	72	10	24	48	72	M x T
Variables									
No. of breasts	12	12	12	12	12	12	12	12	
Time constants (ms)									
T_{2B}	0.76 ± 0.06 ^{ABC}	0.84 ± 0.06 ^{AB}	0.67 ± 0.06 ^{BCD}	1.00 ± 0.06 ^{A}	0.49 ± 0.06^{CD}	0.46 ± 0.06^{D}	0.56 ± 0.06^{BCD}	0.49 ± 0.06^{CD}	<0.01
T_{21}	20.2 ± 1.0 ^{A}	22.5 ± 1.0^{A}	18.8 ± 1.0 ^{AB}	22.5 ± 1.0 ^{A}	13.3 ± 1.0^{C}	12.6 ± 1.01 ^{C}	14.6 ± 1.0^{BC}	13.9 ± 1.0 ^{BC}	<0.05
T_{22}	59.2 ± 6.1	68.6 ± 6.2	49.4 ± 6.2	60.6 ± 6.3	65.0 ± 6.2	77.6 ± 6.3	69.4 ± 6.4	50.6 ± 6.0	0.124
Populations (%)									
T_{2B}	4.63 ± 0.14	4.48 ± 0.14	4.45 ± 0.14	4.31 ± 0.15	5.49 ± 0.14	5.59 ± 0.14	5.05 ± 0.14	5.04 ± 0.13	0.325
T_{21}	86.6 ± 0.5 ^{D}	87.8 ± 0.5 ^{CD}	89.3 ± 0.5 ^{CD}	90.1 ± 0.5 ^{BC}	92.9 ± 0.5 ^{AB}	93.6 ± 0.5 ^{A}	93.7 ± 0.5^{A}	93.5 ± 0.5 ^{A}	<0.05
T_{22}	8.73 ± 0.56 ^{A}	7.66 ± 0.56 ^{AB}	6.22 ± 0.56 ^{AB}	5.57 ± 0.58 ^{B}	1.62 ± 0.6^{C}	0.79 ± 0.6 ^{C}	1.15 ± 0.6 ^{C}	1.48 ± 0.5 ^{C}	<0.05
Hardness									
Compression (N)	26.6 ± 1.2	26.4 ± 1.2	21.9 ± 1.2	24.4 ± 1.2	24.4 ± 1.2	22.2 ± 1.2	24.0 ± 1.2	21.3 ± 1.2	0.057

$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

the cited studies, normal breast meat did not experience a change in the water distribution during ageing (P > 0.05); on the contrary, storage time affected the T_{21} population percentage and that of the T_{22} population in WB breasts. At the same time, an expected association between changes in water compartmentalisation and a more tender meat was not corroborated by the compression results. Indeed, samples analysed at different times and/or characterised by different muscle conditions exhibited a similar hardness (Table 3).

The effect of the interaction between muscle condition and sampling location (M x L) on myowater NMR relaxation times, myowater populations percentages and breast meat hardness is shown in Table 4. Overall, the study of the interaction M x L revealed that the NMR relaxation properties of myowater markedly differed between the two conditions. In WB affected samples, the Location CRA/S possessed the longest T_{2B} and T_{21} relaxation times, contrary to all the other locations. All the four sampling locations of normal breasts had similar T_{2B} and T_{21} relaxation times. The two groups exhibited rather similar T_{22} relaxation times, except for the significant differences (P < 0.01) observed between N MED/D vs N CRA/S, WB CRA/D and WB MED/D.

The overall interaction M x L did not affect the proportion of the T_{2B} population, but on the contrary, the T_{21} and T_{22} populations were significantly influenced. In detail, all the four locations of normal samples had similar T_{21} percentages, which were higher in particular than that of Location CRA/S of WB samples (P < 0.01). On the contrary, in WB group, Location CRA/S had the highest T_{22} population, followed by MED/S and then CRA/D and MED/D together (P = 0.01). Again, concerning T_{22} population percentage, all N Locations had similar and lower values than those of WB (P = 0.01). Globally, Location CRA/S was harder than Locations CRA/D and MED/D, while the differences between CRA/S and MED/S both in WB and N groups were not significant (P > 0.01). Contrary to the expectations based on the study of Soglia et al. (2017), the CRA/S layers of WB and N breasts exhibited the same hardness values.

pH is known to affect the water-holding capacity (Hamm, 1972; Puolanne & Halonen, 2010) as well as firmness of raw meat, although there are not published papers on that subject. The pH difference observed between WB and N breast muscles has been about 0.1–0.2 units (Soglia et al., 2016a), and the value is around 6.0 in modern fast-growing birds, which would mean in other meat animals so called dark cutting meat, but with WB this is not relevant. At this level pH means higher water-holding (Hamm, 1972), which is the contrary to what WB causes in meat. Higher pH means also increased firmness in raw meat like dark-firm-dry beef, but not even close to the extent that would be comparable to the WB hardness of focal WB of young birds, and especially not of diffuse cases of WB (Sihvo et al., 2014; Sihvo et al., 2017b).

Table 4
Effect of the interaction between muscle condition (M) and Location (L) on NMR T_2 relaxation times and population percentages, and hardness. Total number of breasts: 96 (48 WB and 48 N muscles, collected during two samplings). The mean values of each location (CRA/S, CRA/D, MED/S and MED/D), consist in the mean of values obtained at 10, 24, 48 and 72 h *pm*.

Muscle condition (M)	WB				N				*P*-value
Locations (L)	CRA/S	CRA/D	MED/S	MED/D	CRA/S	CRA/D	MED/S	MED/D	M x L
Variables									
No. of samples	48	48	48	48	48	48	48	48	
Time constants (ms)									
T_{2B}	1.23 ± 0.05 ^{A}	0.63 ± 0.04 ^{C}	0.81 ± 0.05 ^{B}	0.60 ± 0.04 ^{C}	0.51 ± 0.04^{C}	0.50 ± 0.04 ^{C}	0.51 ± 0.04 ^{C}	0.49 ± 0.04 ^{C}	<0.01
T_{21}	26.1 ± 0.7 ^{A}	17.9 ± 0.6 ^{C}	23.0 ± 0.6 ^{B}	17.0 ± 0.6 ^{C}	14.1 ± 0.62 ^{DE}	13.1 ± 0.6^{E}	13.8 ± 0.6 ^{E}	13.3 ± 0.6 ^{E}	<0.01
T_{22}	69.8 ± 5.2 ^{AB}	50.1 ± 4.6 ^{B}	66.2 ± 5.0 ^{AB}	51.6 ± 4.6 ^{B}	52.5 ± 4.7 ^{B}	67.7 ± 4.8 ^{AB}	63.5 ± 4.7 ^{AB}	79.0 ± 5.0 ^{A}	<0.01
Populations (%)									
T_{2B}	4.11 ± 0.14	4.79 ± 0.12	4.07 ± 0.13	4.92 ± 0.12	4.95 ± 0.12	5.58 ± 0.13	5.09 ± 0.12	5.55 ± 0.12	0.412
T_{21}	85.0 ± 0.4 ^{D}	90.8 ± 0.4 ^{B}	87.6 ± 0.4 ^{C}	90.6 ± 0.4 ^{B}	93.5 ± 0.4^{A}	93.3 ± 0.4 ^{A}	93.6 ± 0.4 ^{A}	93.3 ± 0.4 ^{A}	<0.01
T_{22}	10.9 ± 0.5 ^{A}	4.42 ± 0.41 ^{C}	8.37 ± 0.44 ^{B}	4.47 ± 0.42 ^{C}	1.57 ± 0.42^{D}	1.10 ± 0.43 ^{D}	1.25 ± 0.42 ^{D}	1.12 ± 0.43 ^{D}	<0.01
Hardness									
Compression (N)	30.9 ± 1.0 ^{A}	20.6 ± 0.9 ^{C}	26.3 ± 1.0 ^{AB}	21.4 ± 0.9 ^{C}	26.3 ± 0.9 ^{AB}	21.0 ± 0.9 ^{C}	23.2 ± 0.9 ^{BC}	21.4 ± 0.9 ^{C}	<0.01

Locations: CRA/S = cranial/surface; CRA/D = cranial/depth; MED/S = medial/surface; MED/D = medial/depth.
$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

4. Conclusions

This study demonstrated that the Wooden Breast condition in meat-type broiler chickens is associated with a different water distribution and myowater properties compared with muscles without Wooden Breast lesion, as indicated by the increased T_2 relaxation times for myofibrillar water and partial water relocalisation to extramyofibrillar spaces in Wooden Breast samples. A connection relating increased muscle hardness and longer relaxation time of water trapped into the myofibrillar matrix was also found; however, an association between changes in water NMR relaxation times and meat hardness was found only for the WB condition and not in Normal meat.

Conflict of interest

The authors declare no conflict of interest.

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